

FELINE PANLEUKOPENIA VIRUS INFECTION IN VARIOUS SPECIES FROM HUNGARY

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Summary

Feline parvovirus (FPV) induced disease in cats has been known since the beginning of the 20th century. Despite initial observations, it has been demonstrated that lions (*Panthera leo*) can also get infected and develop clinical disease. The present work describes the results of the macroscopic, and light microscopic changes induced by FPV and the electron microscopic and genetic demonstration of the infection in several species, as well as the phylogenetic analysis of FPV strains currently circulating in Hungary.

Key words: feline panleukopenia virus, macroscopic and microscopic changes, genetic analysis, Hungary

Feline panleukopenia (FP) is caused by the feline parvo- or panleukopenia virus (FPV), a single stranded DNA virus belonging to the *Parvoviridae* virus family (1). FPV is closely related to other parvoviruses, such as the mink enteritis virus (MEV), raccoon parvovirus (RPV), type 2 canine parvovirus (CPV2) and blue fox parvovirus (BFPV) (2, 3). FPV-induced disease in domestic cats has been known since the beginning of the 20th century (4, 5). Initially the disease was described as “feline infectious enteritis”, “malignant panleukopenia”, “feline distemper” or “spontaneous agranulocytosis” (4, 6). The first outbreaks of the disease in captive felids were reported in the 1930's and 1940's (7, 8).

There are several reports of FPV infections that occurred in wild species affecting tigers (*Panthera tigris*), leopards (*Panthera pardus*), cheetahs (*Acinonyx jubatus*), wild cats (*Felis sylvestris*), lynx (*Lynx lynx*), servals (*Leptailurus serval*), tiger cats (*Felis tigrina*; *Felis aurata*), ocelots (*Leopardus pardalis*), lions (*Panthera leo*), snow leopards (*Panthera unica*), clouded leopards (*Neofelis nebulosa*), mountain lions (*Felis concolor*), African wild cat (*Felis lybica*), leopard cats (*Felis bengalensis*) and maned wolves (*Chrysocyon brachyurus*) (5, 6, 9). Based on the reported cases it is generally assumed that all members of the family *Felidae* are susceptible to FPV infection and disease (6). Raccoons (*Procyon lotor*) and Arctic or blue foxes (*Alopex lagopus*) with clinical signs of a FPV disease were recognized in the 1940's (10, 11). The parvoviruses isolated from these species were described as FPV-like viruses and named after their respective hosts, RPV and BFPV (12, 13). A parvovirus infection of a South American coati (*Nasua nasua*) has also been reported (14). Evidence of the susceptibility of members of the *Viverridae* animal family was represented only by the result of serological testings (9). The epidemiology of FPV is characterized by an acute

infection with shedding of high virus titers in the feces of diseased animals. Virus shedding usually lasts only 1 to 2 days, but virus can stay infectious in the environment for weeks or even months (15). Direct contact between carnivores is not required for efficient transmission (6). FPV is maintained in a population by its environmental persistence rather than prolonged viral shedding; hence fomites play an important role in disease transmission because of prolonged survival of the virus on all sorts of contaminated surfaces, such as shoes, hands, food dishes, bedding etc. (15). The incubation period before the onset of clinical signs is usually 4-5 days, and the clinical course can rapidly progress to death. Due to virus properties and needs, the primary pathologic site for viral replication is within the intestinal crypts, resulting in enteritis and diarrhea due to malabsorption and increased permeability (16). The virus affects cells located deep in the intestinal crypts, while differentiated absorptive cells on the surface of the villi are non-dividing and are not affected. Thymic atrophy and extensive damage to all white blood cell populations and precursors, resulting in severe leukopenia are also common consequences of FPV infections (15).

The aim of the present study was to determine the incidence of FP in Hungary, and to perform the phylogenetic analysis of Hungarian FPV strains.

Materials and methods

Samples were represented by fecal samples obtained from clinically ill patients and tissue samples acquired during necropsy from 33 cats (*Felis catus*), one Asian palm civet (*Paradoxurus hermaphroditus*), and one lion (*Panthera leo*). The analyzed samples were acquired by different departments of the Faculty of Veterinary Science, or they were sent in by veterinary practitioners from different parts of Hungary. The Asian palm civet was submitted by an exotic animal dealer from Budapest, while the lion carcass was sent in for diagnostic purposes by a circus lion tamer from Érd, Hungary. Neither the palm civet nor the lion had received any vaccinations.

Histopathology: tissue samples for the histopathological examination were fixed in 8 % buffered formaldehyde solution for 24 h, embedded in paraffin, then 4 µm thick sections were cut using a sledge type microtome and stained with HE according to the routine methods.

Electron microscopic investigation: fecal samples were suspended 1:3 in distilled water, cleared by low speed centrifugation, followed by 20 min at 9000 x g. The samples were prepared according to the single-droplet negative staining technique (17) and examined at a JEM 1011 transmission electron microscope (JEOL, Japan).

Genetic analyses: samples were homogenized in 10 ml phosphate buffered saline (PBS) and centrifuged at 1500 x g for 10 min. The viral nucleic acid was isolated from the supernatants using the QIAamp viral RNA Mini Kit (Qiagen, Germany) and High Pure Viral Nucleic Acid Kit (Roche, Switzerland), according to the manufacturers' instructions. Phylogenetic analysis was performed using amplicons of several other sets of primer pairs (Table 1), as previously described (18, 19, 20) Following electrophoresis in a 2 % Low Melting Agarose Gel (Bio-Rad Laboratories, USA), the amplicons were cut out

from the gel, and DNA was extracted with the QiaQuick Gel Extraction Kit (Qiagen, Germany). Fluorescence-based direct sequencing was performed in both directions on the amplicons at Biomi Kft. (Gödöllő, Hungary) and Biogon Kft. (Budapest, Hungary), using ABI 3100 genetic analyzers (Applied Biosystems, USA). The nucleotide sequences were identified using the BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>). The two direction sequences were compiled and aligned using the Align Plus 4 software (Scientific and Educational Software, USA) and Clustal X.

Table 1

Primers used for the demonstration and genetic analysis of parvovirus strains

Primer	Sequence 5'-3'	Sense	Position*	Annealing (°C)	Amplicon size (bp)
CPV/FPV diag F	GACTTGTGCCTCCAGGTAT	+	2388-2408	49	420
CPV/FPV diag F	GTTGAACTGCTCCATCACTC	-	2787-2807		
F2	ACCAACTTGGCGTTACTCAC	+	2154-2174	50	1014
R4	GTTGCCAATCTCCTGGAT	-	3149-3167		
CPV2-FPV-F	CACAGCAAACCTCAAGCAGAC	+	2968-2988	48	898
CPV2-FPV-R	GTCTGCTGCAATAGGTGTT	-	3846-3866		
VPM**	TGGAGGTA AACAGGAATT	+	4094-4113	52	437
VPR**	TTTCTAGGTGCTAGTTGAG	-	4512-4531		

*positions are according to the nucleotide sequence of the CPV2 reference strain (accession number NC001539)

**previously described (22)

Phylogenetic trees were constructed using the nucleic acid sequences of the Hungarian CDV, FPV and CPV2 strains, as well as sequences of corresponding lengths acquired from the GenBank (<http://www.ncbi.nlm.nih.gov>) by neighbor-joining with two parameters distance matrix (21), using the Phylip program. The robustness of the groupings in the neighbor-joining analysis was assessed with 100 bootstrap resamplings.

Results and discussions

During necropsy pathological changes suggestive of a parvovirus infection, such as: dehydration, anemia, enteritis and enlargement of the abdominal lymph nodes and of the spleen were observed. In case of the lioness, an extensive bacterial necrotizing myositis, following a traumatic injury that penetrated the skin, could be observed on the left hind limb. Other findings were represented by a penetrating ulcer in the cranial segment of the duodenum and a subsequent serous peritonitis. In case of the Asian palm civet, the most relevant pathological signs were the severe dehydration, firmness of the affected intestinal loops, enlargement of the mesenteric lymph node and of the spleen (Fig. 1).

In all FPV infection cases, regardless of species, multifocal crypt necrosis, moderate mononuclear cell infiltration in the propria, shortening of intestinal villi, signs of regeneration tendencies, lymphoid depletion and the presence of nuclear inclusion bodies could be observed (Fig. 2A). In case of the lioness, an extended

necrotizing myositis in the left hind limb and the penetrating ulceration of the duodenum were also present.

The EM examination of the diluted feces of FPV positive cats and the Asian palm civet revealed the presence of numerous rounded viral particles, measuring about 18 to 21 nm in diameter. Based on their ultrastructural morphology, the viral particles were identified as members of the *Parvoviridae* family (Fig. 2B).

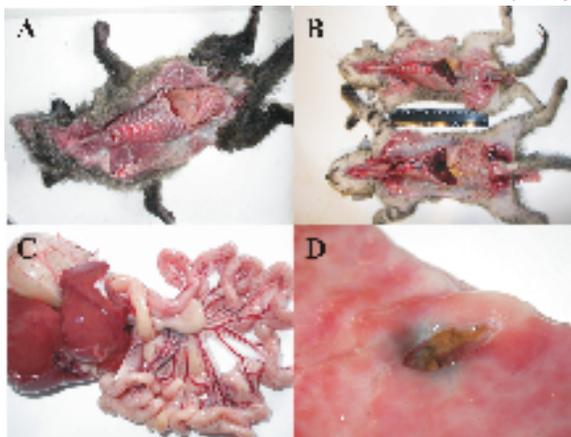


Fig. 1. A: extreme dehydration and anemia in the Asian palm civet; B: carcass of two dehydrated kittens with firm, extremely dilated intestinal loops; C: enlarged mesenteric lymph node and dilated intestinal loops with characteristic, rigid walls (Asian palm civet); D: ulcerative enteritis in the African lion

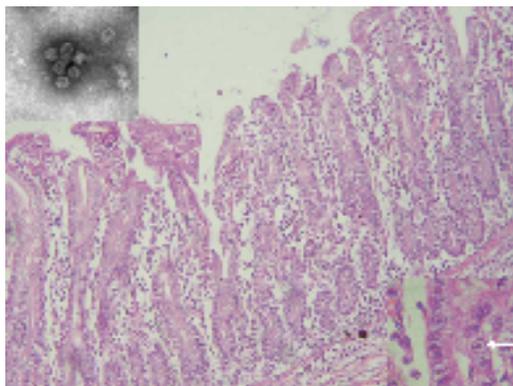


Fig. 2. Intestinal histopathology of the FPV positive Asian palm civet – upper left insert: electron microscopic features of viral particles with a morphology characteristic for parvoviruses; lower right insert: higher magnification with nuclear inclusion body (white arrow) in an epithelial cell (HE staining)

FPV genetic material was successfully demonstrated in 17 out of the 33 examined cats (51.5%), 1 lion, and 1 Asian palm civet (Table 2).

Table 2

FPV positive samples analyzed in the present study			
	Sample code	Description of the animals	Sample type
1	389/07	2 months old, ♂ Asian palm civet	Intestine, feces
2	933/07	1 year old, ♂ domestic cat	Intestine, feces
3	1335/07	7 weeks old, ♂ Persian cat	Intestine, feces
4	1259/08	1.5 years old lioness	Intestine

PCR positive samples from two cats, one lion and one Asian palm civet were selected for phylogenetic analysis. Based on these analyses, all strains turned out to be FPVs. The analysis of the Hungarian FPV strains demonstrated that the virus strain that infected the Asian palm civet is most closely related to other FPV strains such as JF-3 (DQ099431) isolated in China (99.54 %), Gercules Biocentr (AY665655) from Russia (99.54 %) and XJ-1 (EF988660) reported in China (99.48 %), and to an RPV strain (M24005) as well (99.48 %).

In the attempt to clarify the time and place of infection, two Hungarian FPV strains demonstrated in cats (933/07: EU360958 and 1335/07: EU360959) were also included in the genetic analysis. Both strains showed the similar level of identity with strain 389/07 at the nucleic acid level (99.43 %), but they were positioned on different branches of the phylogenetic tree. Strain 933/07 was positioned right next to strain 389/07 and the Chinese isolate JF-3, while strain 1335/07 seemed to be more closely related to Argentinean FPVs (Fig. 3).

The FPV strain demonstrated in the dissected lion showed the highest level of identity with a virus strain isolated in Russia (AY665655: 99.83 %), while on the phylogenetic tree was placed on the same branch with strain 377 (U22188), isolated in Germany.

FPV-induced disease in cats has been known since the beginning of the 20th century (4, 5). There are several wild species from different animal families that are known to be receptive for FPV (6, 23). Up to this point no reports were submitted regarding the direct demonstration of an FPV infection in any member of the *Viverridae* animal family, but one publication reported FPV seropositivity survey in Formosan gem-faced civets (9). The PCRs produced in each FPV infection case amplicons of the expected size, demonstrating the presence of the parvovirus genome. The subsequent genetic analyses demonstrated that the virus strains demonstrated in Hungarian cats and Asian palm civet are closely related and are positioned on the same cluster of the phylogenetic tree (Fig. 3). The virus strain demonstrated in the Asian palm civet was most closely related to other FPV strains such as JF-3 (DQ099431) isolated in China (99.54 %), Gercules Biocentr (AY665655) from Russia (99.54 %) and XJ-1 (EF988660) reported in China (99.48 %), but to an RPV strain (M24005) as well (99.48 %).

Hungarian strains and an Asian virus strain in the same cluster, the genetic analysis of these samples unfortunately did not help to clearly elucidate the time and place of infection. The only indirect evidence is the moment of the onset of clinical signs and eventual death of the animal (approximately 50 hours following its arrival to Hungary). Even though FPV has a very short incubation period (15), based on the severity of the macro- and microscopical findings and on the genetic data available, at this point it can be only assumed that the infection occurred in Malaysia, prior to the animal's arrival to Hungary.

The overall result irrefutably demonstrates the parvovirus infection in the examined animal. The nucleotide sequence of the amplicons resulted following the PCRs have revealed that the causative agent is an FPV strain. On the other hand these investigations revealed that more wild species are susceptible to FPV infections than known before, hence the causative agent has a host range even wider than it has been suspected. It may also mean that more rigorous measures need to be taken such as quarantine and other prophylactic measures when importing wild and exotic species with unknown immunological history in order to prevent infections and the spreading of pathogens. The examinations represented by macroscopical findings, histopathology, EM and especially those based on a PCR technique described, are the first direct diagnosis of an FPV infection in an Asian palm civet and in any member of the *Viverridae* animal family.

Pathological changes observed during the dissection of the analyzed lion were suggestive of a viral infection. The histopathological examination of tissue specimens only emphasized the initial observations. The presence of FPV was demonstrated by the means of diagnostic PCR. All attempts to demonstrate the presence of other pathogens (canine distemper virus, feline coronavirus, feline calicivirus, feline herpesvirus 1, *Chlamydophyla psittaci*) yielded negative results (19). The necrotic myositis present in the hind limb, cerebral edema, ulcerations of the tongue were most likely consequences of the severe immunosuppression caused by FPV. The phylogenetic analysis of the FPV strain that infected the lion revealed that it is most closely related to a German isolate (U22188), and it is positioned on a different branch of the phylogenetic tree than the other Hungarian strains (Fig. 3). These findings seem to emphasize the genetic diversity of Hungarian FPVs, similarly to previous findings (24). The infection of wild and exotic species is unfortunately another evidence for the susceptibility of these species, and the necessity for the implementation of more severe quarantine and prophylactic measures in order to avoid contact of these animals with potential carriers of FPV.

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